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Effect of substrate anchoring on the mechanical strength of Langmuir–Blodgett bilayers

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Abstract

Using the surface-force apparatus (SFA), we have made out-of-contact force and in-contact adhesion measurements between composite Langmuir–Blodgett (LB) bilayers. The outer layer is a crystalline monolayer of a novel glycine-containing amphiphile, whose headgroups make a strong, hydrogen-bond-aided adhesion when in normal contact with an identical monolayer. Two different inner layers were studied, with different affinities for the mica substrate. One, a crystalline monolayer of the phospholipid dipalmitoylphosphatidyl-ethanolamine (DPPE), is held onto mica by comparatively weak interactions. Another monolayer, a covalently polymerized network of octadecyltriethoxysilane (OTE), is covalently anchored to plasma-treated mica through the thermal hydrolysis of silanol groups on both substrate and monolayer. We observe that separation of two DPPE/glycine-amphiphile bilayers in hydrogen-bonded contact leads to fracture at the mica/bilayer interface, with a pull-off force of $F/R = -75$ mN/m. Bonded OTE/glycine-amphiphile bilayers fracture at the OTE/glycine-amphiphile interface, with a pull-off force of $F/R = -142$ mN/m. These results demonstrate that the mechanisms of LB film fracture cannot be explained considering only the breaking of hydrophobic contacts between neighboring amphiphile tails.

Keywords: Surface-force apparatus; Adhesion; Langmuir–Blodgett bilayers

1. Introduction

The Langmuir–Blodgett (LB) technique has for years held the promise of creating highly ordered, molecularly thin, and homogenous layers by a facile methodology [1]. However, the relatively poor thermostability of LB layers has precluded their widespread industrial use [2]. As a result, many investigations have been undertaken to improve the structural integrity of LB layers through the use of bridging counterions [3], membrane-spanning dipolar lipids [4], adsorbed or covalently bound polymers [4], and by polymerization of previously associated amphiphiles [5]. Here, we investigate the effect of strong, covalent attachment of the bilayer to a solid support on the mechanical integrity of the film.

Recently, a protocol was developed whereby LB monolayers are covalently attached to plasma-treated mica using silane chemistry [6]. Octadecyltriethoxysilane (OTE) is spread on an acid subphase in a Langmuir trough, and the OTE is allowed to polymerize into large, two-dimensional islands (~10 μm) of material. The OTE islands are readily transferred to plasma-treated mica, and are held in place by hydrogen-bonding interactions between the silanol groups on the islands and those created on the mica surface by the plasma treatment. These hydrogen-bond dimers undergo hydrolysis at high temperatures, yielding a highly hydrophobic surface stabilized both laterally and normally by covalent silane bonds.

Here, we apply a second LB monolayer to the OTE surface of a novel glycine-containing amphiphile. The glycine headgroup features amide and carbonyl linkages, capable of forming complementary hydrogen bonds with opposed glycine amphiphiles. FTIR spectra of cast films of the glycine amphiphile show wavelength shifts characteristic of hydrogen bonding between these functional groups. Previous work using the surface-force apparatus (SFA) [7] has shown that opposing dipalmitoylphosphatidyl-ethanolamine (DPPE)/glycine-amphiphile bilayers on mica make a strong, hydrogen-bond-aided adhesive contact. Any attempt to separate the bonded bilayers tears them from the mica surface to maintain headgroup contact. The adhesion is extinguished at high pH, as charging of the headgroups prevents the formation of inter-layer hydrogen bonds.

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In this work, we compare the adhesive strength and mode of fracture of identical bilayers of DPPE/glycine-amphiphile and OTE/glycine-amphiphile on mica using the SFA. This gives us a direct comparison of the effect of covalent anchoring on the mechanical integrity of LB bilayers.

2. Experimental details

All glassware and Teflon used in this experiment was cleaned using Chromerge cleaning solution (Fisher Chemical). All stainless steel, including the SFA itself, was cleaned using 50% nitric acid (Mallinckrodt). Both were removed by copious rinsing with MilliQ-purified water (Millipore).

The glycine amphiphile (Fig. 1) was synthesized using a previously reported protocol [8]. Briefly, the sixteen-carbon dialkyl tail was created by acid-catalyzed condensation of an hexadecanol with l-glutamic acid (Sigma). The resulting ester was then reacted with succinic anhydride (Sigma). The carboxylic-acid terminus of this intermediate was activated with *p*-nitrophenol (Sigma), a good leaving group for reaction with OBz-protected amino acids (Bachem). Protecting groups were removed by hydrogenation over platinum catalyst.

Langmuir isotherms and LB deposition were carried out using an automated LB trough (KSV Instruments). LB bilayers were deposited onto cylindrically curved glass lenses with a 1 cm^2 mica coupon glued to them. The lenses were held by stainless-steel tweezers for the deposition. DPPE (Avanti Polar Lipids) was spread from a 1 mg/ml solution in 99:1 chloroform/methanol (Mallinckrodt) onto a subphase of MilliQ-purified water. DPPE was transferred on the upstroke at 1 mm/min at a surface pressure of $\pi = 41$ mN/m (crystalline phase). The lenses were allowed to dry in a clean airstream for 15 min. The glycine amphiphile was spread from a 1 mg/ml solution in chloroform on the same subphase, and transferred on the downstroke at 1 mm/min at a surface pressure of $\pi = 35$ mN/m (liquid-condensed phase). Transfer ratios were 1.00 ± 0.02 in both cases.

OTE was spread from a 2 mg/ml solution onto a subphase of pH 2 nitric acid. Island formation was allowed to occur for 30 min, after which the islands were compressed to $\pi = 25$ mN/m for deposition. Deposition substrates were mica-covered lenses which were exposed to a 30 W argon/water-vapor plasma (Harrick) at 57 Pa for 2 min. Deposition of OTE islands was performed on the upstroke at 1 mm/min, with a transfer ratio of 0.95 ± 0.05. The lenses were annealed in a clean dessicator at 80°C for 60 min. After cooling, the glycine amphiphile monolayer was applied as before, with a transfer ratio of 0.87 ± 0.05.

In both cases, the bilayer-covered lenses were transferred using small beakers from the subphase of the LB trough to a Mark II SFA filled with MilliQ-purified water for force measurements. The operation of the SFA has been reported elsewhere [9]. Briefly, micro-Newton forces between the lenses are measured by the deflection of supporting springs, and angstrom-level separation distances are determined by a multiple-beam interferometric technique. Force versus distance profiles measured between the two lenses are scaled by the radius of curvature of the lenses (R), yielding a quantity (F/R) proportional to the energy of interaction per unit area between flats. The force required for pull-off (F) is calculated from the spring deflection measured just prior to separation. For comparison purposes, this quantity is also scaled by the radius of curvature (F/R). The separation distance (D) was set to zero at bare mica/bare mica contact for the data presented here.

AFM images were obtained using a MultiMode™ nanoscope III AFM unit provided by Digital Instruments. OTE/

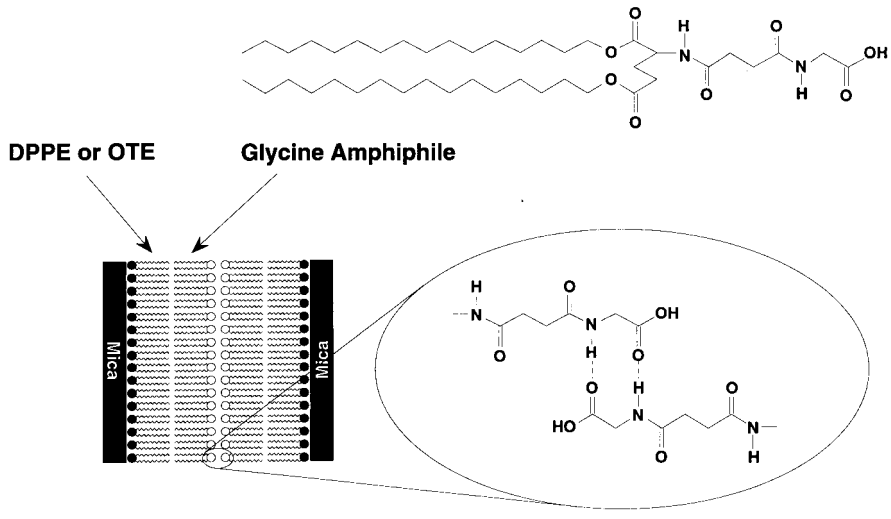

Fig. 1. Structure of the glycine amphiphile and its position in the LB bilayers. The inset depicts the inter-layer hydrogen bonding established in adhesive contact.

glycine-amphiphile bilayers were transferred under water from the LB trough to a fluid cell for imaging.

3. Results and discussion

3.1. Langmuir isotherms

Fig. 2 shows the Langmuir isotherms measured during the creation of DPPE, OTE, and glycine amphiphile monolayers. Deposition of DPPE was performed at 41 mN/m, well into the crystalline regime, where the area/molecule equals the area/molecule of two hydrocarbon tails (0.4 mm^2). The isotherm for the OTE islands shows a small expanded region, suggesting that a considerable amount of unpolymerized material remains between the polymerized islands as the layer is compressed [6]. This material should act to hydrophobize the inter-island spaces, shielding the surface charge of mica. A highly stable crystalline phase is observed above 20 mN/m, at a molecular area equivalent to the cross-sectional area of a single hydrocarbon tail (0.2 nm^2) [10].

Deposition of the glycine amphiphile was performed at 35 mN/m, in the liquid-condensed regime. At this region of the isotherm the amphiphiles are very closely packed, with a molecular area near 0.4 nm^2. The glycine head groups, in the LB bilayers deposited at this pressure, should not possess a great deal of mobility.

3.2. AFM images of OTE monolayers and OTE/glycine-amphiphile bilayers

Fig. 3a is a contact-mode AFM image of the annealed OTE islands on mica. The islands are roughly circular with a diameter of about 10 μm. A section analysis (inset) has the OTE islands 14 Å higher than the inter-island spaces. Since the OTE molecule is about 28 Å long [11], we would expect a height difference of 28 Å if the inter-island spaces were bare mica. These spaces are probably covered by unpolymerized material, as suggested by the isotherm data.

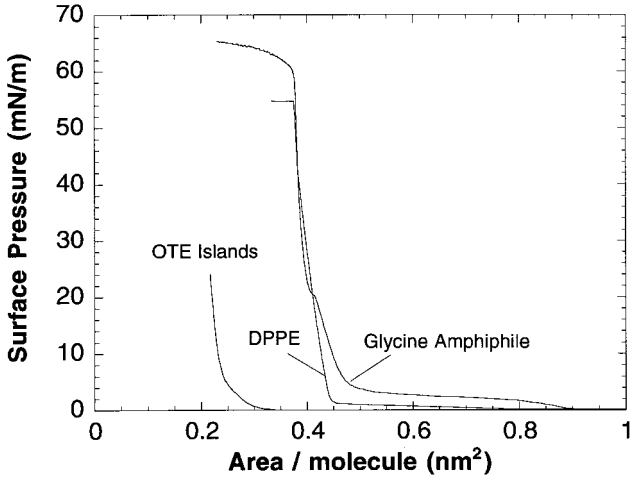

Fig. 2. Langmuir isotherms of DPPE and the glycine amphiphile on a pure water subphase (pH 6.8). 'OTE islands' refers to the isotherm obtained during the compression of polymerized OTE on a pH 2 HNO_3 subphase.

The contact-mode image of the OTE/glycine-amphiphile bilayer (Fig. 3b) shows that the glycine amphiphile failed to transfer onto some regions of the OTE monolayer, which accounts for the slightly lower transfer ratio observed during the deposition. The section analysis of the inter-island spaces of the bilayer has a height difference of 55 Å, equal to the length of the glycine amphiphile (30 Å [11]) plus the depth of the inter-island spaces in the OTE monolayer (14 Å). During dipping, defects are nucle-ated in the glycine amphiphile monolayer as the dipping front encounters the inter-island spaces. These defects progressively shrink as the transfer front moves to a new island, leading to the triangular defects observed in Fig. 3b. These defects, while interesting, constitute about 10% of the total bilayer surface as judged by the glycine-amphiphile transfer ratio. We expect these defects to contribute little to the measured force and adhesion data, which is averaged over an area of about 100 μm^2.

3.3. Forces between DPPE/glycine-amphiphile bilayers

Force versus distance profiles for the interaction of identical DPPE/glycine-amphiphile bilayers in 1 mM KBr (pH 6.8) are shown in Fig. 4. On first approach (filled circles), the bilayers repel each other electrostatically beginning at a separation distance of 500 Å. This repulsion increases exponentially to a separation distance of 125 Å. The bilayers jump into a strongly adhesive contact at a separation distance of D_o = 102 Å, marked by a pronounced flattening of the FECO interference fringes used to measure the separation distance in the SFA experiment [12]. This value corresponds to the thickness of the two opposed bilayers, and agrees well with a simple space-filling model that has an anhydrous bilayer thickness of 58 Å [10,11]. The value we measure is slightly less than 58 Å × 2 = 116 Å, owing to the slight interpenetration that is required for the formation of complementary hydrogen bonds (Fig. 1). A fairly large pull-off force (F/R = -75 mN/m) was required to separate the bonded bilayers.

The force data are readily fitted to a DLVO expression [10], accounting for van der Waals attraction and electrostatic repulsion between the layers. A Hamaker-constant expression was used for the van der Waals portion of the interaction:

$$\left.\frac{F}{R}\right|_{VDW} = -\frac{A}{6D^2}$$

where the non-retarded Hamaker constant (A) was set to 7×10^{-21} J, an experimentally obtained value for bilayers of the phospholipid DPPC [9].

The electrostatic portion is described by a constant-charge solution of the non-linear Poisson–Boltzmann ex-

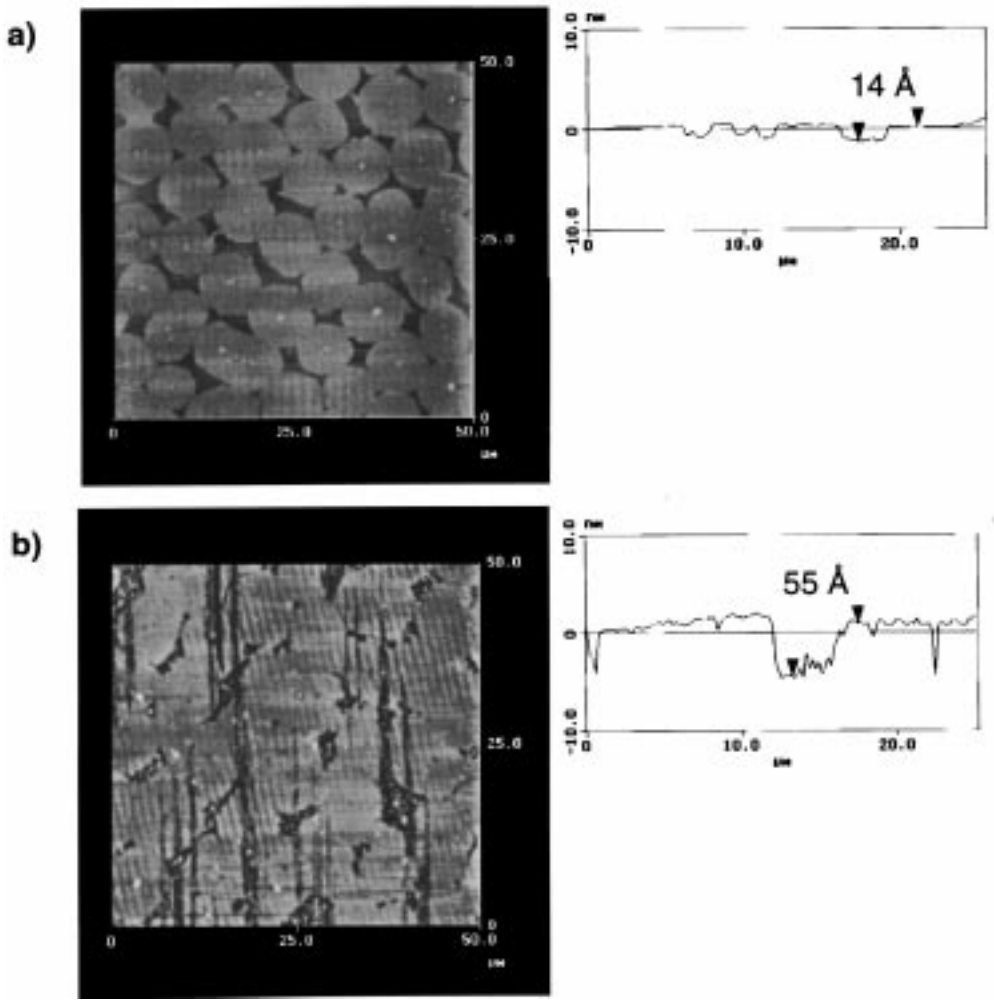

Fig. 3. AFM images and section analyses for (a) partial monolayer of annealed OTE on plasma-treated mica in air, and (b) a similar monolayer after LB deposition of glycine amphiphile in water.

pression [13], with a Debye screening length of 90 Å and a surface potential of −125 mV. The fitted Debye length agrees well with that expected for a solution of 1 mM KBr (100 Å). Using the Grahame equation [14], the surface charge density can be deduced from the fitted surface potential. Given the density of lipids deposited, we conclude that 5.1% of the lipids at the surface carry a charge, most likely at the carboxylic acid terminus. Previous work has shown that these LB layers [7] and SAM's bearing terminal carboxylic acids [15] have half the achievable carboxylic acid dissociation completed at a pH between 7.0 and 7.5. This represents a substantial increase from the pK_a of the carboxylic acid of free glycine (2.3 [16]), owing to surface confinement effects [17]. The origin of DLVO forces ('Outer Helmholtz Plane', OHP) was set at a separation distance 15 Å greater than the contact separation distance (D_o) to account for the slight interpenetration of headgroups on contact (Fig. 1).

On a second approach, a similar repulsive force is measured up to 220 Å, at which point a steep steric repulsion appears. The repulsion is relieved at 20 mN/m, giving way to a second steep repulsion at 157 Å. The bilayers could not be brought back to the original contact separation distance of 102 Å, even at very high loads ($F/R > 100$ mN/m). Since these repulsions appear at approximately one and two bilayer thicknesses from the original contact, we believe these are steric repulsions brought about by the uprooting of bilayer domains from the surface of mica to maintain hydrogen bonds between glycine-amphiphile headgroups [18].

The fracture of these bilayers at a hydrophobic surface, rather than at the hydrogen-bond-linked headgroup interface cannot be explained based solely on energetic arguments. The free energy required to break these hydrogen bond pairs is much smaller than that for the transfer of an individual lipid from a hydrophobic matrix to water [19]. Similar bilayer 'uprooting' has been reported in adhesion measurements between streptavidin-crosslinked biotin-conjugated bilayers [20,21]. It has been argued that the uprooting occurs as a series of energetically inexpensive 'slips', in which only a few methylene groups are exposed to water at a time [21,22]. If this process sets the pull-off force, then

anchoring the lower layer should decrease its value, as fewer 'slips' are required for the bilayers to separate.

3.4. Forces between OTE/glycine-amphiphile bilayers

Force versus distance profiles for the interaction of identical OTE/glycine-amphiphile bilayers in 1 mM KBr (pH 6.8) are shown in Fig. 5. On first approach, these bilayers exhibit the same electrostatic repulsion observed for the DPPE/glycine-amphiphile bilayers. The DLVO fit has a Debye length of 90 Å, again in good agreement with that expected for the ionic strength used (100 Å). The fitted surface potential of −125 mV is identical to that for the DPPE/glycine-amphiphile bilayers. These observations confirm that the surfaces of these bilayers behave similarly in these measurements, and the only observable difference between the two is the manner in which they are anchored to mica.

These bilayers jump into a strongly adhesive contact at a separation distance of 108 Å, marked by a pronounced flattening of the FECO interference fringes used to measure separation distance in the SFA experiment [12]. In some cases, the flat region showed a small indentation; the separation distance calculated at the depth of the indentation was 10 ± 2 Å smaller than the remainder of the contact region. This indentation indicates the presence of an inter-island space inside the measurement area. Because measurements made with and without the indentation yield identical force versus distance data, we do not believe that the inhomogeneity of the bilayers (Fig. 3) plays a significant role in these measurements.

A pull-off force of $F/R = -142$ mN/m was required to separate the OTE/glycine-amphiphile bilayers, nearly twice that required to separate the DPPE/glycine-amphiphile bilayers. On second approach, a force curve similar to the initial one is observed up to a separation distance of 170 Å, only one bilayer thickness from the initial contact.

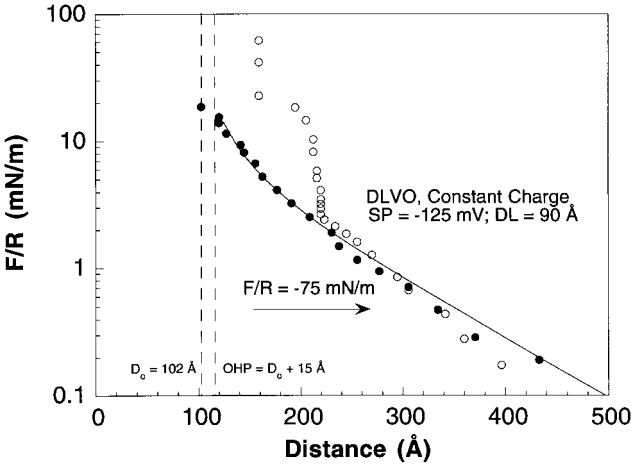

Fig. 4. SFA force curves for the interaction of DPPE/glycine-amphiphile bilayers in 1 mM KBr (pH 6.8). Filled circles are first approach data, open circles are second approach data. The line is a fit to DLVO theory (see text).

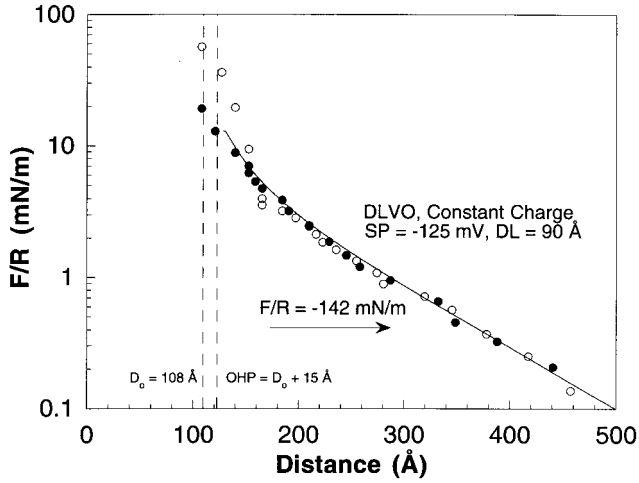

Fig. 5. SFA force curves for the interaction of OTE/glycine-amphiphile bilayers in 1 mM KBr (pH 6.8). Filled circles are first approach data, open circles are second approach data. The line is a fit to DLVO theory (see text).

Furthermore, these bilayers can be brought back to the original contact thickness at a modest applied load ($F/R = 50$ mN/m). Apparently only parts of the outer leaf were removed, since only one small steric repulsion is observed on second approach. The covalent anchoring of the OTE monolayer successfully held it in place during the separation of the hydrogen-bonded bilayers.

The higher force required to separate these surfaces cannot be explained by the sequential 'slipping' model described above. Fewer 'slips' should certainly be required to pull out a single monolayer of material than a bilayer, and therefore, an equal or lower pull-off force should be measured. It seems a larger force is required to initiate a slipping event for the OTE bilayers, and the initiation sets the magnitude of the pull-off force. This is reasonable, since in the case of DPPE the bonded bilayers prefer to separate at the headgroups rather than at the bilayer midplane. The DPPE headgroups are held on mica by fairly weak charge interactions as best, and some water trapped at the interface during the deposition may further weaken this interaction. Parting two purely hydrophobic surfaces in water should be more energetically expensive.

Another possibility is that domains of amphiphiles are removed from the bilayers, and the domain size is not the same in each case. This explanation is also reasonable, since the OTE islands are large and polymerized, while DPPE grain boundaries are smaller and stabilized by weaker forces. Efforts to strengthen these bilayers should focus on reducing the number of layer defects, should this be true.

4. Conclusions

We have demonstrated that the structural integrity of supported bilayers can be dramatically improved by covalently anchoring the bilayer to the substrate. Hydrogen-

bonded DPPE/glycine-amphiphile bilayers fracture at the headgroup/mica interface on separation. OTE/glycine-amphiphile bilayers, with the same surface chemistry but covalently anchored to mica, fracture at the midplane of the bilayers. It requires nearly twice as much force to achieve this fracture, a result which cannot be explained considering only hydrophobic interactions inside the bilayers. These results should be considered in the design of increasingly robust LB films.

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